

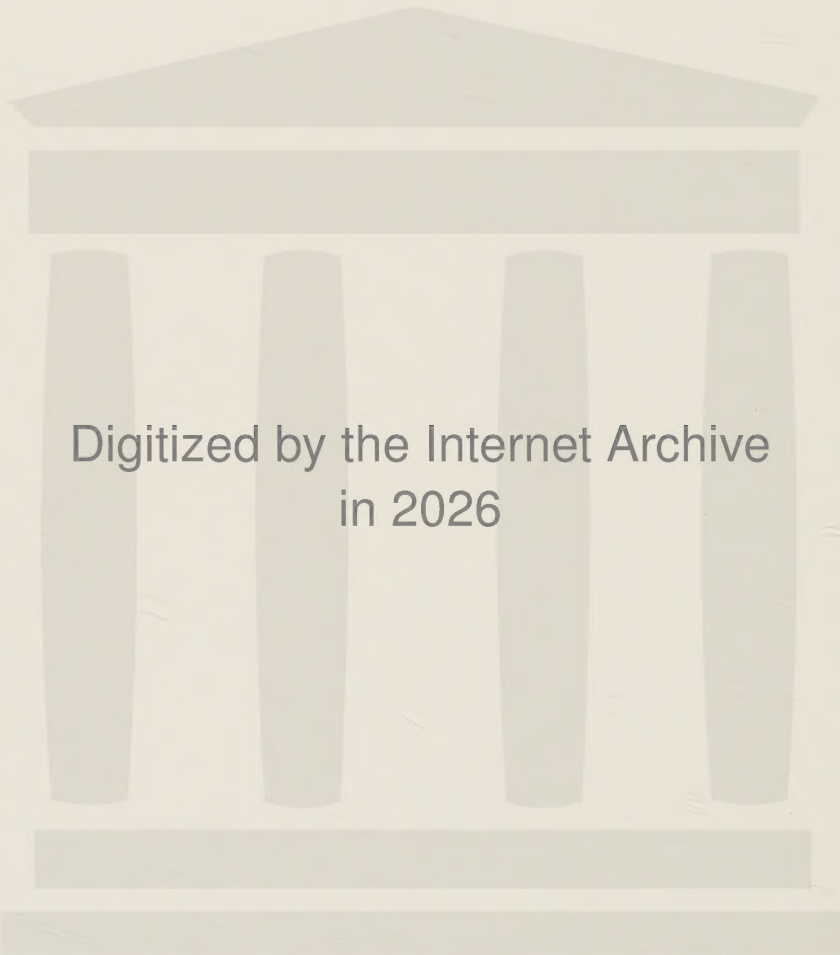
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**GENETICS OF HUMAN
PLACENTAL PHOSPHATASES**

BY

GUNHILD BECKMAN

UMEA 1970



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GENETICS OF HUMAN PLACENTAL PHOSPHATASES

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AV

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GENETICS OF HUMAN PLACENTAL PHOSPHATASES

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TO
LARS

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P R E F A C E

The present thesis is a summary of a series of publications which are listed below. Results published in these publications will be referred to by roman numerals in the text. The thesis does also contain some previously unpublished data.

- I. *L. Beckman, G. Beckman, C. Christodoulou and A. Ifekwunigwe*: Variations in human placental alkaline phosphatase. *Acta genet. (Basel)* 17: 406—412 (1967).
- II. *G. Beckman and E. O. Jóhannsson*: Distribution of placental alkaline phosphatase types in the Icelandic population. *Acta genet. (Basel)* 17: 413—417 (1967).
- III. *L. Beckman and G. Beckman*: Individual and organ-specific variations of human acid phosphatase. *Biochem. genet.* 1: 145—153 (1967).
- IV. *L. Beckman, G. Beckman, S. Bergman and E. Lundgren*: Isozyme variations in human cells grown in vitro. II. Acid phosphatase. *Acta genet. (Basel)* 18: 409—415 (1968).
- V. *L. Beckman and G. Beckman*: A genetic variant of placental alkaline phosphatase with unusual electrophoretic properties. *Acta genet. (Basel)* 18: 543—552 (1968).
- VI. *L. Beckman, G. Beckman and M. P. Mi*: The relation between human placental alkaline phosphatase types and some perinatal factors. *Hum. Hered.* 19: 258—263 (1969).
- VII. *G. Beckman and L. Beckman*: The placental alkaline phosphatase polymorphism; variations in Hawaiian subpopulations. *Hum. Hered.* 19: 524—529 (1969).
- VIII. *G. Beckman, L. Beckman and A. Tärnvik*: A rare subunit variant shared by five acid phosphatase isozymes from human leukocytes and placenta. *Hum. Hered.* 20: 81—85 (1970).
- IX. *G. Beckman*: Placental alkaline phosphatase; relation between phenotype and enzyme activity. *Hum. Hered.* 20: 74—80 (1970).
- X. *G. Beckman and L. Beckman*: Relation between the ABO blood groups and the level of placental alkaline phosphatase in sera of pregnant women. *Hum. Hered.* 20: preprint (1970).
- XI. *G. Beckman, L. Beckman and E. Lundgren*: Isozyme variations in human cells grown in vitro: IV. Identity between alkaline phosphatases from HeLa cells and placenta? *Hum. Hered.* 20: preprint (1970).

INTRODUCTION

During the last decade a considerable amount of information has accumulated concerning genetic enzyme variations in Man (*cf.* Harris, 1969). This development is largely due to the introduction of new methods of electrophoretic separation which have facilitated the discrimination of multiple molecular forms of enzyme proteins (isozymes or isoenzymes). The concept of the isozyme was introduced by Markert and Møller in 1959 in order to describe the existence of two or more kinds of molecules which catalysed the same biochemical reaction but differed in their electrophoretic properties. Isozymes differ very often not only electrophoretically but also in a number of other physico-chemical properties (*cf.* Vesell, ed. 1968). There has been some confusion as to the exact meaning of the term isozyme. In this work the classification into primary and secondary isozymes proposed by Shaw (1969) has been adopted. Thus primary isozymes are distinctly different protein molecules presumably controlled by different genetic loci, while secondary isozymes are electrophoretically distinct enzyme components resulting from secondary changes in the enzyme molecule. Most secondary isozymes appear to be artifacts and hence probably of little biological significance. Primary isozymes may also show electrophoretically distinct variants controlled by allelic genes.

Isozymes may often show organ-specific and ontogenetic variations, which indicate that they may play a significant role in the processes of development and differentiation. Placental isozymes are of specific interest in this context, since recent results have shown that they may be controlled by the foetal genotype. (Robson and Harris, 1965).

Alkaline phosphatase (E. C. 3. 1. 3. 1.) and acid phosphatase (E. C. 3. 1. 3. 2.) of the placenta have been the subject of many histochemical studies (Velardo and Rosa, 1963). The specific activity of alkaline phosphatase in placental tissue increases during pregnancy and reaches its maximum just prior to term, while the specific activity of acid phosphatase on the other hand decreases slightly toward the end of pregnancy (McKay et al., 1958). Alkaline phosphatase in the placenta is an organ-specific isozyme (Boyer, 1963; Sussman et al., 1968) while acid phosphatase in the placenta exhibits three isozymes with a general somatic distribution and one isozyme which is foetal, but not strictly placental (III). The purposes of this investigation were:

1. to identify isozymes and isozyme variants of placental alkaline and acid phosphatase.
2. to analyze the distribution of placental phosphatase isozyme variants in the major ethnic groups of the world.
3. to study the statistical relationship between placental alkaline phosphatase phenotypes and a series of factors relevant to foetal survival.
4. to examine qualitative and quantitative variations of placental phosphatases in the sera of pregnant women and newborn children.
5. to investigate the identity between placental phosphatases and the phosphatase isozymes occurring in some fibroblastic cell strains and epithelial cell lines.

MATERIALS AND METHODS

Materials

1. Samples of placental tissue were obtained from the following sources:

Sweden (Uppsala)	n = 221 (I)
Sweden (Uppsala)	n = 998 (VI)
Sweden (Umeå)	n = 1067 of which 400 were included in (IX)
Iceland	n = 128 (II)
Greece	n = 212 (I)
Nigeria	n = 133 (I)
Hawaii	n = 1505 (VII)

105 twin pairs (with clearly separated placentae) collected from eight hospitals in central Sweden during 1965—1967. Legal abortions (n = 22) collected at the university hospitals in Lund and Umeå, Sweden.

2. Serum samples from mothers and newborns (cord serum) in 400 deliveries from the University Hospital, Umeå, Sweden (IX, X).
3. Specimens of organs obtained from autopsies of adult individuals (III), and specimens of foetal organs from legal abortions (IV).
4. Fibroblastic cell strains established from skin biopsies and epithelial cell lines obtained from the laboratory of professor Sven Bergman, Department of Clinical Bacteriology, Umeå, Sweden (IV, XI).

5. Blood samples (200 ml) from different individuals ($n = 17$), (VIII).
6. Medical records (VI, VII).

Methods

Descriptions of methods used have been given in the following publications:

1. Preparations of extracts from:
 - Placenta and other organs (I, III, IV).
 - Cultured cells (IV, VIII, XI).
 - Leukocytes (VIII).
2. Culturing of cells (IV, XI).
3. Electrophoresis:
 - Buffer systems for the separation of alkaline phosphatase (I, V) and acid phosphatase (III, VIII).
 - Supporting media (I, V).
 - Staining procedures for alkaline phosphatase (I) and acid phosphatase (III, VIII).
4. Assay method for alkaline phosphatase (IX).
5. Heat inactivation of:
 - Alkaline phosphatase (IX).
 - Acid phosphatase (III).
6. Inhibition of:
 - Alkaline phosphatase by L-phenylalanine (XI).
 - Acid phosphatase by L-tartrate (III).
7. Neuraminidase treatment (III, XI).
8. Immunological methods (III, XI).
9. Ultracentrifugation (V).
10. Computer analysis (VI).

PLACENTAL ALKALINE PHOSPHATASE

Placental alkaline phosphatase is distinct from other human alkaline phosphatase isozymes in for example its electrophoretic mobility (Boyer, 1961, 1963), immunological reaction (Boyer, 1963, Sussman et al., 1968), heat-stability (McMaster et al., 1964) and sensitivity to inhibition by L-phenylalanine (Fishman et al., 1965).

Common variants

The existence of a polymorphism in placental alkaline phosphatase was first proposed by Boyer (1961) and verified by Robson and Harris (1965) and Beckman et al. (1966). Robson and Harris (1965, 1967) reported that most placentae could be classified into six common phenotypes if the electrophoretic separation was performed at two different pH's, 8.6 and 6.0. The common phenotypes were called F_1 , F_1S_1 , S_1 , S_1I_1 , F_1I_1 and I_1 . Five of these phenotypes were also described by Beckman et al. (1966). Fig. 1 shows the six common phenotypes of placental alkaline phosphatase after separation on starch gel electrophoresis at pH's 8.2 and 5.6. The electrophoretic mobility of the I_1 variant is decreased to a mobility indistinguishable from that of the S_1 variant when examined at an acid pH. The F_1S_1 , F_1I_1 and S_1I_1 types of placental alkaline phosphatase all show a triple-banded electrophoretic pattern. The zone of intermediate mobility apparently represents a hybrid enzyme suggesting that placental alkaline phosphatase is a dimer and that the hybrid enzyme is formed by a process of random combination of monomers (Robson and Harris, 1965; Beckman et al., 1966). The fact that placental alkaline phosphatase is a dimer has been

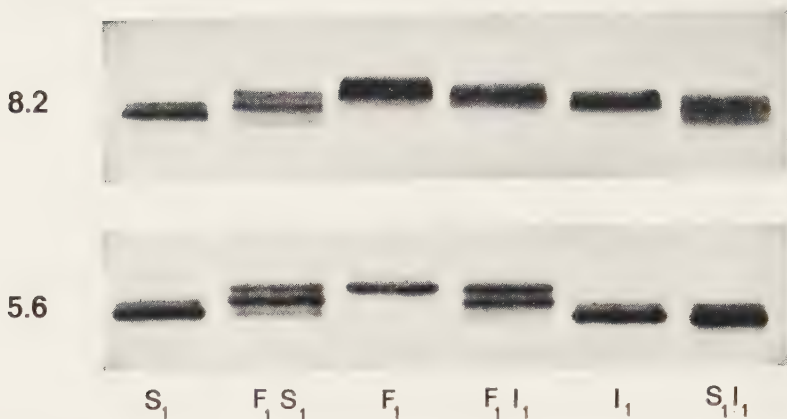


FIG. 1. Photograph of starch gels showing the six common phenotypes of placental alkaline phosphatase. The separation is performed at pH 8.2 and 5.6. The anode is at the top of the picture. The cathodal parts of the gels have been cut off.

verified by Gottlieb and Sussman (1968). They were able to purify placental alkaline phosphatase and to separate the enzyme into two monomers each having the same molecular weight.

Robson and Harris (1965, 1967) studied the alkaline phosphatase phenotypes in a series of altogether 400 twin pairs. The alkaline phosphatase types were found to be controlled by the genotype of the foetus. The results showed a good agreement with the assumption that the six common phenotypes were controlled by three autosomal alleles (Plf_1 , Pls_1 and Pli_1), and that the triple-banded phenotypes were heterozygotes. A series of 100 twin pairs showing the common phenotypes have been examined in our laboratory. A complete diagnosis of zygosity was not attainable, but since all twin placentae were dichorionic (separate) most of the pairs (about 93 %) should be dizygotic (*cf.* Corney et al., 1968). Seventy pairs were concordant and thirty were discordant with respect to placental alkaline phosphatase phenotype (Table 1). On a null-hypothesis of random (non-genetic) pairing of types the expected numbers of concordant and discordant twin pairs would be 34:66. The difference between observed and expected numbers is statistically highly significant ($P < 0.001$).

TABLE 1. *Placental alkaline phosphatase phenotypes in 100 twin pairs.*

Like pairs			Unlike pairs		
Twin combination		No.	Twin combination		No.
S ₁	S ₁	36	S ₁	F ₁ S ₁	14
F ₁ S ₁	F ₁ S ₁	21	S ₁	F ₁	2
F ₁	F ₁	3	S ₁	S ₁ I ₁	1
S ₁ I ₁	S ₁ I ₁	8	S ₁	F ₁ I ₁	1
F ₁ I ₁	F ₁ I ₁	2	F ₁ S ₁	F ₁	1
			F ₁ S ₁	S ₁ I ₁	4
		70	F ₁ S ₁	F ₁ I ₁	3
			S ₁ I ₁	F ₁ I ₁	4
					30

A search for linkage between the Pl locus and sex, ABO, MN, Rh, Kell, Lutheran, Duffy, Kidd, G-6-PD, AK, Ac. Ph. and PGM gave negative results (Edwards and Wingham, 1967).

Rare variants

A number of rare variants of placental alkaline phosphatase have been described (Boyer, 1961, Beckman et al., 1966, I, V, VII, Robson and Harris, 1967, Blake et al., 1968, Blake et al., 1969 a and b). Robson and Harris (1967) introduced the addition of subscripts in order to facilitate the classification of rare variants. In the present investigation eleven rare variants distributed on eighteen rare phenotypes were found (Fig. 2). Three of the variants (F₂, F₃ and S₂) and four of the phenotypes (F₁F₂, F₂I₁, F₃S₁ and S₁S₂) were previously described by Robson and Harris (1967). The following rare phenotypes were not found: F₁S₂, F₁S₄, F₁S₅ and I₁S₃ (Robson and Harris, 1967); "Chinese" (Blake et al., 1968); "Fast Thai" and "Slow Thai" (Blake et al., 1969 a) and "Japanese" (Blake et al., 1969 b). The following notations are suggested for the eight new rare alkaline phosphatase variants found in the present investigation: F₄, F₅, F₆, F₇, S₆, S₇, D₁ and D₂. The rare phenotypes showed tripled-banded (heterozygous) patterns with two exceptions: F₃ and S₆, which thus may be homozygotes for rare genes.

A far better separation of the rare phenotypes was obtained with the buffer system by Ashton and Braden (1961) compared to that

by Poulik (1957). This improved separation is the main reason for the discrimination of the four new F-variants. Electrophoresis at pH's 6.0 and 5.6 gave essentially the same result.

The D-variants differed from all the other rare variants with respect to the electrophoretic patterns found in the heterozygotes. When the D-variants were found together with a common variant the following characteristic deviations were found on starch gel electrophoresis: a) at pH 8.2 the hybrid enzyme did not show a strictly intermediate electrophoretic mobility; it was faster than expected for the D₁-variant and much slower than expected for the D₂-variant. b) at pH 5.6 no triple-banded pattern was found. There were two zones, of which one corresponded to the common variant and the other apparently to the D-variant. Altogether 26 placentae showing the D₂-variant have been found, of which 11 were F₁D₂ and 15 S₁D₂ (V). There is strong evidence that the phenotypes displaying the D₂-variant represent heterozygous combinations between a common allele at the PI locus and a rare allele controlling the D₂-variant. The D₂-variant has about the same molecular weight

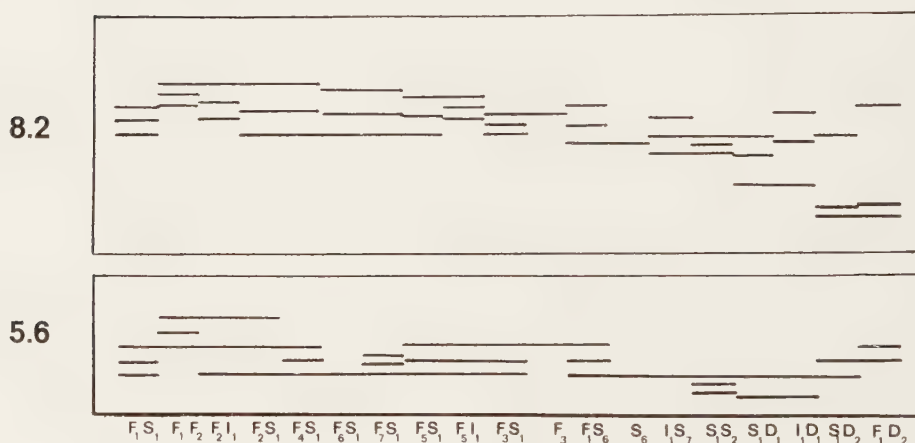


FIG. 2. Schematic picture showing the 18 rare phenotypes of placental alkaline phosphatase found in the present investigation. The F₁S₁ phenotype is included for comparison. The anode is at the top of the picture and the pH's are 8.2 and 5.6. Phenotypes observed previously by other investigators are underlined.

as the other placental alkaline phosphatase variants, and the very slow electrophoretic mobility at pH 8.2 was found only when electrophoresis was performed in starch gel (V). In media like paper or polyacrylamide the D₂-variant had an electrophoretic mobility intermediate between those of the F₁ — and S₁ variants at pH 8.2. The same mobility was found with starch gel electrophoresis after the addition of EDTA to the placental extract or to the gel buffer (final concentration of EDTA 0.01 M and pH of the gel 8.1). The effect of EDTA was abolished if an excess of Zn⁺⁺ (0.1 M ZnCl₂) was added to the EDTA gel. A similar effect was obtained with Co⁺⁺, but not with cations such as Mg⁺⁺ or Ca⁺⁺. The very slow mobility of the D₂-variant on starch gel at pH 8.2 is apparently caused by factors other than the molecular weight and the electric charge of the molecule.

Secondary isozymes

On starch gel electrophoresis each major alkaline phosphatase zone is associated with a series of minor zones, two with a faster mobility and one with a somewhat slower mobility than that of the major zone. These minor zones parallel the electrophoretic differences shown by the main components (Robson et al., 1965, 1967; Beckman et al., 1966). The number and the strength of the minor zones were increased when electrophoresis was performed at pH 5.6. A series of alkaline phosphatase zones with a very slow anodal mobility have been described, which also show electrophoretic variations parallelling those of the major enzyme zones (Boyer, 1961; Robson and Harris, 1965; Beckman et al., 1966). The slow moving components can not be demonstrated when electrophoresis is performed in media such as paper, agarose or polyacrylamide (V). The slow moving enzyme components have apparently about the same molecular weight as that of the major components (Boyer, 1963; V). The minor zones and the slow moving zones are dependent on technical factors and therefore they fit the criteria of "secondary isozymes" proposed by Shaw (1969).

Phenotype and enzyme activity

Robson and Harris (1967) suggested that the Pl_i-allele might contribute less to the heterozygous phenotype than the Pl_f₁- and Pl_s₁-

alleles. The evidence was based on observed deviations from the expected 1: 2: 1 distribution of the relative strength of the zones in the triple-banded electrophoretic pattern of the heterozygote. Their observation has been confirmed. Thus in the F_1I_1 phenotype the F_1 -component and the hybrid enzyme stained more strongly, while the I_1 -component was considerably weaker. (IX).

Quantitative determinations of the placental alkaline phosphatase level in placental extracts and in the serum of pregnant women showed that the Pl_{I_1} -allele was associated with a lower enzyme activity ($P < 0.001$) than that of the Pl_{f_1} - and Pl_{s_1} -alleles (IX). In the placentae of individuals heterozygous for the Pl_{I_1} -allele (F_1I_1 and S_1I_1) the alkaline phosphatase activity was 22 per cent lower than in placentae from individuals lacking the Pl_{I_1} -allele (F_1 , F_1S_1 and S_1). When serum from pregnant women at term was examined the Pl_{I_1} -heterozygotes showed 27 per cent lower activity. Thus one dose of the Pl_{I_1} -allele lowered the enzyme activity with about 25 per cent and the activity attributable to the Pl_{I_1} -allele was estimated to about half that of the Pl_{f_1} - and Pl_{s_1} -alleles.

Alkaline phosphatase in placentae and chorionic tissue from early stages of pregnancy

Placentae and chorionic tissue from 22 legal abortions were examined. The length (crown-rump) of the foetuses varied between 3 and 20 cm corresponding to a prenatal age of about 9—19 weeks of gestation (Boyd, 1941). In some of the smaller foetuses the placental alkaline phosphatase phenotypes showed a rather weak staining, but with the aid of butanol extraction (Morton, 1954) they could be determined in all the cases. In the extracts of chorionic tissue from four small foetuses (length below 10 cm) an extra alka-

TABLE 2. *Distribution of placental alkaline phosphatase phenotypes in a series of placentae from legal abortions (length of foetus less than 20 cm). Obs. = observed numbers. Exp. = expected numbers calculated from the gene frequencies in a material of 2295 Swedes.*

	F_1	F_1S_1	S_1	S_1I_1	F_1I_1	I_1	Rare types	No.
Obs.	3	11	6	0	2	0	0	22
Exp.	1.44	7.27	9.19	2.46	0.97	0.16	0.50	21.99

line phosphatase component was found. This component was heat-sensitive and had a mobility similar to that of liver alkaline phosphatase.

The distribution of placental alkaline phosphatase phenotypes is shown in Table 2. The gene and phenotype frequencies in the fetal series were compared with those of a control series consisting of the combined Swedish materials from Umeå and Uppsala. The expected numbers were calculated on the basis of this control material. The frequency of the Plf_1 -allele is high in the foetal series (0.4318) compared to that in the control material of newborns (0.2556). There is a significant overrepresentation of the Plf_1 gene ($0.01 > P > 0.001$) and of phenotypes carrying the F_1 -factor ($0.05 > P > 0.01$) in the foetal series.

PLACENTAL ACID PHOSPHATASE

Isozymes

In water extracts of placental tissue four electrophoretically distinct zones with acid phosphatase activity were found after starch gel electrophoresis (III). An improved resolution was later obtained by electrophoresis in Tris-borate buffer pH 7.5 (VIII). Extraction with 1 % Triton X-100 did not reveal any additional acid phosphatase zones but gave a blurred appearance of the isozymes and a somewhat stronger staining of the B zone. The four acid phosphatase isozymes were called A, B, C and D in decreasing order of mobility towards the anode (*cf.* Figs. 3, 5 and 7). The A isozyme is usually rather weak and sometimes difficult to detect. Zones corresponding to the A, B and D isozymes were found in extracts of organs other than the placenta (III, IV).

The C isozyme was not observed in the following organs: heart, intestine, kidney, liver, skeletal muscle, skin and pancreas obtained from postmortem examinations of 14 adult individuals, but was always present as a strongly staining zone in placental extracts (III). In 50 out of 53 samples of foetal tissue an acid phosphatase zone with the mobility of the C isozyme was found (IV). The C components were rather strong in extracts of foetal liver, of variable strength in extracts of kidney and lung and faint in skin and intestine (IV).

Isozyme variants

No electrophoretic variations between individual placentae were found in the A- and B isozymes. Four individual placentae with

a deviating electrophoretic pattern were observed (III, VIII). In the position of the C isozyme two zones were found, of which the one with faster anodal mobility corresponded to the normal C isozyme (Fig. 3). In the position of the D isozyme three zones were found, of which the one with the fastest anodal mobility corresponded to the normal D isozyme. The results indicated that the C- and D isozymes were sharing a common polypeptide subunit. The parents of a child with the deviating placental acid phosphatase phenotype were examined with respect to the electrophoretic patterns of their leukocyte acid phosphatases (VIII). The results indicated that the child had inherited a rare gene from her father, which controlled a polypeptide subunit with a slow anodal mobility. This subunit was apparently shared by five different acid phosphatases; the C- and D isozymes, two isozymes found in granulocytes and one isozyme occurring in lymphocytes.

Acid phosphatase in placentae and chorionic tissue from early stages of pregnancy

Placentae and chorionic tissue from the 22 legal abortions mentioned earlier were examined with respect to acid phosphatase isozymes. All extracts showed the same isozyme pattern as in placentae at term, but the staining intensity of the B-, C- and D isozymes was generally much stronger.

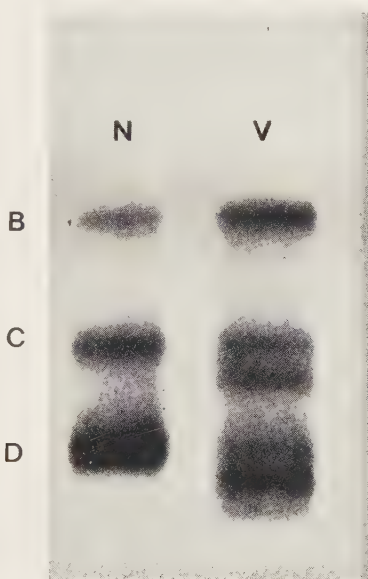


FIG. 3.
Acid phosphatase patterns of a normal placenta (N) and a placenta showing electrophoretic variations in the C and D isozymes (V). The anode is at the top of the picture. pH 8.2.

PLACENTAL PHOSPHATASE ISOZYMES IN THE SERA OF PREGNANT WOMEN AND NEWBORN CHILDREN

Alkaline phosphatase

Fig. 4 shows the electrophoretic mobilities of the alkaline phosphatase isozymes found in the sera of normal adults, pregnant women and newborn children. Three common placental alkaline phosphatase phenotypes are included for comparison. Intestinal alkaline phosphatase is found in a low frequency in the sera of pregnant women (about 10 %) and newborn children (about 1 %) (Beckman and Grivea, 1965). The level of the heat-sensitive liver alkaline phosphatase is normal in pregnant women at term (X).

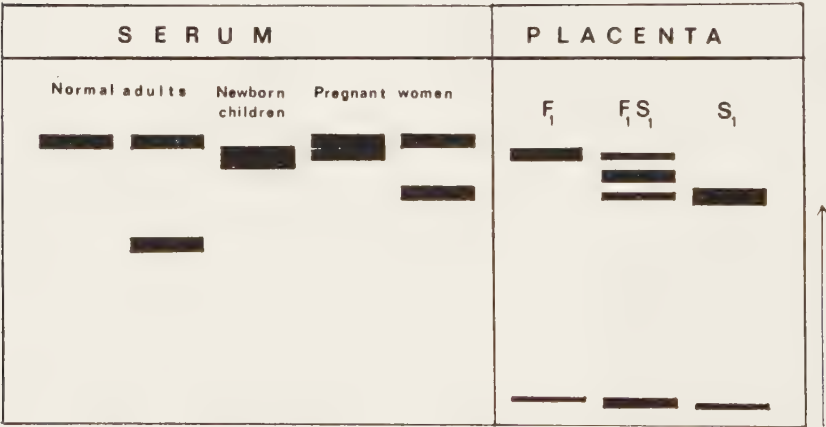


FIG. 4. Alkaline phosphatase isozymes observed in sera of normal adults, newborn children and pregnant women after electrophoresis at pH 8.2. Three common phenotypes of placental alkaline phosphatase are shown for comparison.

An alkaline phosphatase isozyme with a somewhat slower electrophoretic mobility than that of the liver isozyme was found in the sera of newborn children (Boyer, 1961; Beckman and Grivea, 1965). This isozyme is most likely bone alkaline phosphatase (*cf.* Hodson et al., 1962).

Placental alkaline phosphatase is only exceptionally found in the sera of newborn children (Beckman and Grivea, 1965), but is regularly present in the sera of pregnant women at term (Boyer, 1961; Beckman and Grivea, 1965; Beckman et al., 1966). In our material of sera from 400 pregnant mothers detectable levels were found in all the cases, and placental alkaline phosphatase constituted on the average 60 per cent of the total alkaline phosphatase level at the end of gestation (IX, X). After heat-treatment (56° C for 30 min), which removed the activity of all other alkaline phosphatase isozymes, the six common phenotypes of placental alkaline phosphatase could be demonstrated in most serum samples by means of starch gel electrophoresis (IX).

Bardawil (1969) reported that the placental alkaline phosphatase activity was lower in the sera of mothers with blood group O than in those with other blood groups. In our material of sera from about 400 pregnant women at term no statistically significant association was found between the placental alkaline phosphatase level and the ABO blood groups of mother or child (X).

There is a strong effect of placental alkaline phosphatase phenotype on the activity of placental alkaline phosphatase in the sera of pregnant women at term (IX). Other factors may also affect this activity thereamong the placental weight (Table 3). The variation

TABLE 3. *Relation between placental weight (in grams) and the activity of placental alkaline phosphatase in the mothers' serum. Activity expressed as micromoles per ml per hr.*

Placental weight classes	M $\pm$ S. E.	S. D.	No examined
Below 400	6.13 $\pm$ 1.51	6.02	16
400—500	7.14 $\pm$ 0.66	5.05	58
500—600	7.81 $\pm$ 0.32	3.49	128
600—700	8.82 $\pm$ 0.30	2.43	67
Above 700	8.77 $\pm$ 0.51	2.61	26
Total	7.90 $\pm$ 0.22		295

between placental weight classes is statistically significant (variance ratio 2.80, $0.025 > P > 0.01$). The standard deviation (S.D. in Table 3) decreases with increasing placental weight.

Acid phosphatase

Fig. 5 shows the electrophoretic mobilities of the acid phosphatase isozymes found in the sera of pregnant women and newborn children. The acid phosphatase pattern of the placenta is included for comparison. The slowly moving serum isozyme showed in general a stronger activity in the sera of newborn children than in those of the pregnant women. Pregnant women showed an isozyme pattern similar to that observed previously in normal adults (Beckman and Zoschke, 1968). The electrophoretic mobilities of the two acid phosphatase isozymes found in the sera of pregnant women and newborn children did not correspond to any of the placental acid phosphatase isozymes. The placental acid phosphatase isozymes are thus apparently not occurring as serum enzymes.

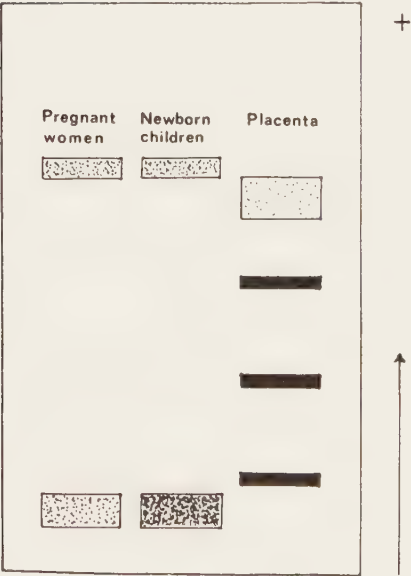


FIG. 5.
Acid phosphatase isozymes in placental extract and in sera from pregnant women and newborn children. pH 8.2.

Gene frequencies

Three alleles controlling placental alkaline phosphatase (Plf_1 , Pli_1 and Pls_1) have been found in rather high frequencies in population samples from various ethnic groups. The main ethnic groups show characteristic distributions of the three common placental alkaline phosphatase alleles (see Table 4). The Plf_1 -allele had its highest frequencies (24—28 %) among Caucasians, while considerably lower frequencies were found among Asiatic mongoloid groups (2—10 %) and African Negroes (2—5 %). The Pli_1 -allele was relatively common among Asiatic mongoloid groups, especially Chinese and Japanese (20—24 %), but lower among Caucasians and Negroes (4—9 %). The Pls_1 -allele is the most common allele in all ethnic groups. The frequency is about 62—68 % in Caucasians, 70—80 % in Asiatic Mongoloids and 90—93 % in African Negroes.

The series of rare alleles described have mostly shown frequencies well below 1 %. The only exception is the Pls_2 -allele (2.9 % among Indians and 1 % among Chinese). The discrimination of some of the types is highly dependent on the resolution of the electrophoretic technique, therefore comparisons should only be made between investigations where the same technique has been employed. Table 5 gives the distribution of rare alleles in the population samples examined by us. In our investigations the D_2 -variant was not observed among Asiatic mongoloids. It was not reported in other investigations of mongoloid groups (Blake et al., 1968, 1969 a and b). The difference between Caucasians and Asiatic mongoloids with respect to the frequency of the Pld_2 -allele is statistically sig-

nificant ($0.01 > P > 0.001$). The Pld_2 -frequency in the series from North Sweden (Umeå) was lower ($0.01 > P > 0.001$) than in that from Central Sweden (Uppsala). No other statistically significant differences were found for the rare Pl -alleles.

All population samples examined with respect to placental alkaline phosphatase were also screened for variations in placental acid phosphatase isozymes. Four cases with a deviating phenotype were found in the Swedish population, which corresponds to a gene frequency of 0.00087.

TABLE 4. *Frequencies of the three common alleles Pl^f1 , Pl^i1 and Pl^s1 in various population groups.*

Population	Ref.	Gene frequency			Rare alleles combined	No examined
		Pl^f1	Pl^i1	Pl^s1		
Icelanders	II	0.2813	0.0313	0.6836	0.0039	128
Swedes (Uppsala)	I, VI	0.2424	0.0808	0.6649	0.0119	1219
Swedes (Umeå)	6	0.2704	0.0934	0.6250	0.0111	1076
English	1	0.2697	0.0854	0.6365	0.0084	597
Greeks	I	0.2844	0.0403	0.6659	0.0096	211
Italians (Rome)	2	0.2558	0.0756	0.6686	*)	185
Caucasians (Hawaii)	VII	0.2902	0.0878	0.6073	0.0145	205
Japanese (Hawaii)	VII	0.0532	0.2471	0.6954	0.0043	348
Japanese (Japan)	3	0.0375	0.2355	0.7236	0.0034	293
Chinese (Hawaii)	VII	0.0224	0.1716	0.7985	0.0075	67
Chinese (Malaysia)	4	0.0297	0.2104	0.7475	0.0124	202
Filipinos (Hawaii)	VII	0.0783	0.1627	0.7590	0.0000	83
Malays (Malaysia)	4	0.1090	0.1209	0.7583	0.0118	211
Thais (Thailand)	5	0.0811	0.1649	0.7460	0.0081	185
Indians (England)	1	0.1961	0.1078	0.6863	0.0098	51
Indians (Malaysia)	4	0.1524	0.0738	0.7429	0.0310	210
Polynesians (Hawaii)	VII	0.1087	0.1087	0.7826	0.0000	23
Negroes (Nigeria)	I	0.0189	0.0379	0.9356	0.0076	132
Negroes (England)	1	0.0556	0.0427	0.8932	0.0086	117

*) = No information available.

References:

1. Robson and Harris, 1967.
2. Bottini et al., 1968.
3. Blake et al., 1969 b.
4. Blake et al., 1969 a.
5. Blake et al., 1968.
6. This report.

Hardy-Weinberg equilibrium

In our investigations (I, II, VI and VII) a good agreement was generally found between the expected and observed numbers of the six common phenotypes (Table 6). The rare phenotypes were omitted in these calculations.

In the total mixed population of Hawaii there was a highly significant deviation from the Hardy-Weinberg distribution characterized by an excess of homozygotes and a deficiency of heterozygotes. About the same type of deviation was found in the mixed Caucasian-Oriental-Polynesian group. The total Swedish material also showed a significant deviation from the expected distribution, but the type of deviation was different from that found in Hawaii and thus not consistently reflecting an effect of inbreeding.

Associations with placental weight and abortion

The relation between placental alkaline phosphatase phenotypes and a series of factors of potential importance for foetal health and survival were examined in a series of deliveries from Uppsala, Sweden (VI). The perinatal factors were: age of mother, birth order, sex of child, gestation length, birth weight, placental weight, previous abortions and blood groups (ABO and Rh) of mother. The most common factor S_1 was found to be associated with a lower placental weight ($0.05 > P > 0.01$) and a lower rate of previous abortion ($0.05 > P > 0.01$). When the frequency of abortions in previous pregnancies was recalculated omitting primiparae the same result as in the previous analysis (VI) was found. In mothers with placentae lacking the S_1 -factor the frequency of previous abortion was 0.1521, while in mothers with placentae having the S_1 -factor the frequency was 0.0946. The difference is significant ($0.05 > P > 0.01$). The major contribution to the high rate of abortions in the S_1 -negative group (F_1 , F_1I_1 and I_1) comes from the F_1 -phenotype. The frequency of this phenotype among newborn children whose mothers had previous abortions (0.1157) is significantly ($P = 0.01$) higher than that of the control series (0.0542).

TABLE 6. Observed and expected numbers of the six common phenotypes in some populations assuming a Hardy-Weinberg equilibrium.

Population		F ₁	F ₁ S ₁	S ₁	S ₁ I ₁	F ₁ I ₁	I ₁	$\chi^2_{3d.f.}$	No
Icelanders	Obs.	12	43	64	3	5	0	4.69	127
	Exp.	10.2	49.3	59.6	5.5	2.3	0.1		
Swedes	Obs.	169	741	959	280	78	17	10.41 ^{*)}	2244
	Exp.	149.1	757.7	962.4	256.6	101.0	17.1		
Greeks	Obs.	20	74	98	8	5	2	6.84	207
	Exp.	17.4	80.4	92.9	11.1	4.8	0.3		
Caucasians (Hawaii)	Obs.	21	63	82	21	10	2	2.53	199
	Exp.	16.6	71.7	77.3	21.8	10.1	1.5		
Japanese (Hawaii)	Obs.	0	26	173	109	11	26	2.71	345
	Exp.	1.0	25.8	167.7	119.9	9.2	21.4		
Chinese (Hawaii)	Obs.	0	3	44	15	0	4	1.82	66
	Exp.	0.0	2.4	42.6	18.5	0.5	2.0		
Filipinos (Hawaii)	Obs.	1	8	51	16	3	4	2.40	83
	Exp.	0.5	9.9	47.8	20.5	2.1	2.2		
Negroes (Nigeria)	Obs.	0	5	116	8	0	1	0.34	130
	Exp.	0.0	4.9	114.9	9.8	0.2	0.2		
Mixed Caucasian Oriental-Polynesian group (Hawaii)	Obs.	8	41	159	63	14	15	11.05 ^{*)}	300
	Exp.	4.2	49.9	148.4	75.2	12.7	9.5		
Mixed total population (Hawaii)	Obs.	42	210	779	331	56	66	43.30 ^{***)}	1484
	Exp.	20.6	247.5	742.2	367.1	61.1	45.4		

^{*)} = 0.05 > P > 0.01.
^{***)} = P < 0.001.

PLACENTAL PHOSPHATASE ISOZYMES IN SOME HUMAN CELL STRAINS AND CELL LINES

In this thesis the cell culture terminology of Hayflick (Hayflick and Moorhead, 1961) has been adopted as the information necessary for a classification according to the nomenclature proposed by the Tissue Culture Association (Fedoroff, 1967) was not available.

Alkaline phosphatase

The alkaline phosphatase levels are quite variable among both fibroblastic cell strains and epithelial cell lines (*cf.* Cox and MacLeod, 1962). Organic phosphates are known to increase the alkaline phosphatase activity of growing human fibroblastic cultures (Cox and Pontecorvo, 1961). In some human epithelial cell lines alkaline phosphatase was induced (3-to 20-fold) by the addition of hydrocortisone or its analogue prednisolone to the culture media, while the alkaline phosphatase levels of human fibroblasts were unaffected by these steroids (Cox and MacLeod, 1961, 1962).

Cox and MacLeod (1962) found that many of their fibroblastic strains did not contain any alkaline phosphatase when grown under standard conditions. In our laboratory we were unable to demonstrate a distinct isozyme pattern in most fibroblastic cell strains. A single weakly staining alkaline phosphatase isozyme was found in human skin fibroblasts, and this enzyme component did not correspond to any of the isozymes found in the HeLa cell lines Ch and S₃ (Cox and Griffin, 1967).

Some HeLa cell lines show a heat-stable, L-phenylalanine sensitive alkaline phosphatase isozyme which is inducible by means of prednisolone (Griffin and Cox, 1966). Fishman et al., (1968)

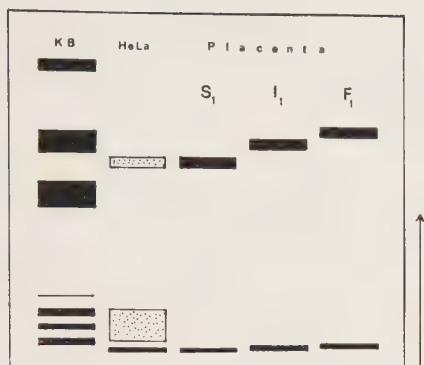


FIG. 6.
Alkaline phosphatase isozymes in the KB and HeLa cell lines compared with the three common variants of placental alkaline phosphatase. pH 8.2.

pointed to the similarities between this heat-stable alkaline phosphatase of HeLa cells and placental alkaline phosphatase. In our investigations evidence has been presented that the heat-stable alkaline phosphatase of HeLa cells is identical with the S₁-variant of placental alkaline phosphatase (XI). Fig. 6 shows the electrophoretic pattern of alkaline phosphatase in a HeLa cell line investigated by us. Extracts of the KB cell line and placentae of the three common phenotypes are shown for comparison. The HeLa cells showed three zones, of which one was wide and diffuse. The two other zones coincided electrophoretically with the fast- and slow moving components of the S₁-phenotype when the electrophoresis was performed at pH's varying between 8.6 and 5.6. Other properties like heat-stability, presence of sialic acid, L-phenylalanine sensitivity and antibody reaction were in agreement with the hypothesis that the alkaline phosphatase of the HeLa cells was placental alkaline phosphatase. KB is an example of an epithelial cell line showing a series of alkaline phosphatase isozymes, which are quite different from those of the HeLa cells.

Acid Phosphatase

Acid phosphatase activity is readily detectable in both fibroblastic cell strains and in epithelial cell lines. In skin extracts components with the electrophoretic mobility of the B isozyme and sometimes the A isozyme were found (IV). When skin explants were cultured

the differentiation into fibroblasts was regularly accompanied by the appearance of a component with the mobility of the D isozyme (Fig. 7). In a number of fibroblast cultures (four out of seventy) an acid phosphatase zone with the electrophoretic mobility of the C-isozyme was found. These cells showed an acid phosphatase pattern which was indistinguishable from that of the placenta. In epithelial cell lines A, B and D zones were observed, but no C-component (IV). Otherwise there were no consistent differences between fibroblastic cell strains and epithelial cell lines.

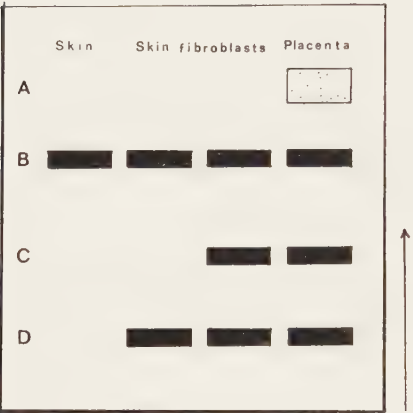


FIG. 7.
Acid phosphatase isozymes in skin, skin fibroblasts and placenta. pH 8.2.

PYROPHOSPHATASE ACTIVITY OF PLACENTAL ALKALINE PHOSPHATASE

Placental alkaline phosphatase as well as alkaline phosphatases from other organ sources are known to hydrolyse a great number of various substrates but have previously been regarded as devoid of activity on substrates as inorganic pyrophosphate (Ahmed and King, 1960) and nucleotide pyrophosphates (Morton, 1955). Results have recently been presented however, which indicate that alkaline phosphatase also possess pyrophosphatase activity.

Russell (1965) reported a patient with congenital deficiency of serum alkaline phosphatase, who exhibited an increased urinary excretion of inorganic pyrophosphate. This observation led to the suspicion that inorganic pyrophosphate might be a physiological substrate for alkaline phosphatase. This hypothesis has lately been verified by a number of workers (Cox and Griffin, 1965; Moss et al., 1966; Fernly and Walker, 1966; Moss et al., 1967; Eaton and Moss, 1967 a and b; Butterworth, 1968; Sussman and Laga, 1968; Griffin, 1969). The ability of alkaline phosphatase to act on a number of organic di- and tripyrophosphates of physiological significance has also been reported (Fernly and Walker 1966; Eaton and Moss, 1967 c; Griffin, 1969). Sussman and Laga (1968) found in contrast to Ahmed and King (1960) that purified placental alkaline phosphatase possessed inorganic pyrophosphatase activity. The alkaline phosphatase activity (substrate: p-nitrophenyl phosphate) had a pH optimum of 11.5, while the inorganic pyrophosphatase activity showed a pH optimum at 8.5.

Moss et al. (1967) used "the lead conversion method" according to Allen and Hynick (1963) to demonstrate alkaline phosphatase activity after separation of the enzymes from extracts of liver and

intestine on starch gel electrophoresis. They found identical isozyme patterns when α -naphthyl phosphate and inorganic pyrophosphate were used as substrates.

In this investigation the ability of placental alkaline phosphatase to hydrolyse different substrates containing pyrophosphate bonds were tested by means of the zymogram technique. A series of common and rare variants were examined by starch gel electrophoresis at pH 8.2. The starch gel was divided lengthwise, one slice was incubated in α -naphthyl phosphate and the other slice in the substrate to be tested. The gels were incubated for 6 h at 37° in Tris HCl buffer pH 8.5. The substrate concentration was 5 mM for inorganic pyrophosphate and thiamine pyrophosphate, and 2 mM for other substrates. Magnesium chloride (1 mM) and lead nitrate (3.3 mM) were added to the stain mixture and after the incubation the zones were developed by the addition of 0.2 % ammonium sulphide.

If the two starch gel slices showed corresponding enzyme patterns it was concluded that the substrate in question was hydrolysed by placental alkaline phosphatase. All placental alkaline phosphatase isozyme variants were found to hydrolyse the following substrates containing pyrophosphate bonds: inorganic pyrophosphate, thiamine pyrophosphate, adenosine di- and triphosphates (ADP and ATP), uridine di- and triphosphates (UDP and UTP). Fig. 8 shows the



FIG. 8.

Starch gel electrophoresis showing three common phenotypes S_1 , F_1S_1 and F_1 stained for pyrophosphatase activity. The substrate is inorganic pyrophosphate. pH 8.2.

electrophoretic patterns of three common placental alkaline phosphatase phenotypes stained with inorganic pyrophosphate as substrate. There is usually no difficulty in identifying the placental alkaline phosphatase phenotypes with the pyrophosphate substrates listed above provided that the concentration of magnesium chloride is correct. Attempts to demonstrate pyrophosphatase activity by the placental acid phosphatase isozymes were unsuccessful.

DISCUSSION

Isozymes and differentiation

Placental alkaline phosphatase is an organ-specific, foetal isozyme controlled by a gene, which normally is active only in placental cells. There is evidence, however, that placental alkaline phosphatase may be derepressed in cancer cells (Fishman et al., 1968) and in epithelial cancer cell lines *in vitro* (XI), while it has not been found in fibroblastic cell strains (Cox and Griffin, 1967).

Acid phosphatase of the placenta shows a situation different from that of placental alkaline phosphatase. Three of the four acid phosphatase isozymes (A, B and D) have apparently a rather general somatic distribution while one (isozyme C) is found in placenta and in other foetal organs (III, IV). The C-isozyme is thus foetal, but not placenta-specific. Components corresponding to the A, B and D isozymes have been observed in both fibroblastic cell strains and in cancer cell lines. An isozyme corresponding to the foetal C-isozyme was found in some fibroblastic cell strains derived from human adult skin, but has not been found in epithelial cancer cell lines (IV). Thus it is possible that the C-isozyme sometimes may be derepressed in fibroblastic cell strains.

The mechanisms controlling the derepression of placental alkaline phosphatase and acid phosphatase C in normal development and in cultured cells are unknown. It seems reasonable to assume, however, that the adjustment of the cell to the transition from an *in vivo* to an *in vitro* situation involves the activation of a different set of genes. In the normal differentiation and development of the human placenta both placental alkaline phosphatase and acid phosphatase C appear very early. These isozymes were observed in

chorionic tissue from fetuses of about 3 cms of length corresponding to a developmental stage of about the 9th week of gestation.

Genetic control

The genetic control of the common placental alkaline phosphatase phenotypes has been demonstrated beyond doubt by means of twin studies. By inference it seems likely that also the rare phenotypes are controlled by the Pl-locus and for some of the rare phenotypes evidence for a genetic control has been obtained by the finding of concordant twin pairs. Of particular interest is the observation of a dizygous twin pair with separate placentae concordant for both placental alkaline phosphatase deficiency and for Dysostosis cranio-facialis (Crouzon). Both placental alkaline phosphatase deficiency and the Crouzon syndrome are very rare and the occurrence together in a dizygous twin pair indicates a possible causal relationship. The observation is furthermore suggesting that there may exist a rare "silent allele".

The genetic background of the rare acid phosphatase phenotype is not as well founded as that of the placental alkaline phosphatase phenotypes. No information was attainable by the analysis of twin placentae because of the rareness of the phenotype. The fact that the placental acid phosphatase isozymes C and D seemed to share a polypeptide subunit with some acid phosphatase isozymes from leukocytes made it possible to obtain some information from the study of leukocyte acid phosphatases of the parents. The child had apparently inherited from her father a gene which controlled a polypeptide subunit found in five different acid phosphatase isozymes thereamong the placental C- and D-isozymes.

Variations in serum

The placental phosphatases show marked differences with respect to their occurrence in serum. None of the placental acid phosphatase isozymes were found in the serum of either mother or child. Placental alkaline phosphatase on the other hand is normally found in the serum of the pregnant mother (Boyer, 1961; Beckman and Grivea, 1965), where it constitutes on the average 60 % of the total alkaline phosphatase activity at the end of gestation (IX). The enzyme may

also occasionally be found in the serum (cord serum) of the child (Beckman and Grivea, 1965) though in a comparatively low concentration. The reason for this occurrence in the sera of only a few children is unknown. So is the nature of the barrier between the maternal and foetal circulations with respect to placental alkaline phosphatase.

Bardawil (1969) presented results which indicated that the serum level of placental alkaline phosphatase was significantly lower in mothers with blood group O. We were unable to conform this result (X) when testing a series of serum samples which was considerably larger than that examined by Bardawil (1969). Conclusive evidence for an association between ABO blood group and serum alkaline phosphatase has been obtained only for the intestinal isozyme (X). The effect of placental alkaline phosphatase phenotype on the level of the enzyme in serum (and placental extracts) is highly significant and of such a magnitude that it should be taken into consideration if measurements of heat-stable alkaline phosphatase are to be used in clinical practice as a criterion of placental function (*cf.* Messer, 1967). Other factors e. g. the placental weight may also influence the serum level of placental alkaline phosphatase.

Nothing is known about the immunological consequences of the particular foeto-placental-maternal interaction displayed by placental alkaline phosphatase. From an immunological point of view a considerable number of mother — child combinations are potentially incompatible insofar that many mothers are carrying in their circulation high levels of a foetal-placental enzyme protein which is incompatible with their own genotype. The compatibility of mother — child combinations can not be established for obvious reasons hence studies of the relation between compatibility and enzyme level in serum are not feasible. Another way to approach the problem would be to search systematically for antibodies in the serum of pregnant women. Such studies are under way.

Polymorphism

The Pl-locus shows a remarkable degree of multiple allelism; 33 different phenotypes have been described, corresponding to 17 different alleles. Placental acid phosphatase shows a striking contrast to the variability at the Pl-locus. In a series of about 3,500 pla-

centae only four had a deviating acid phosphatase phenotype. Only three of the genes controlling placental alkaline phosphatase would with certainty satisfy the criteria of a polymorphism (Ford, 1945). The Pls_2 -allele in Indians and Chinese represents a border-line case (Blake et al., 1968). Whether the Pls_2 -allele is polymorphic or not can not be judged with certainty due to technical limitations. Variants with a similar electrophoretic mobility may be difficult to discriminate, and in such cases the gene frequency estimate may actually be a sum of the frequencies of two or more genes and consequently too high. If the rare alleles at the Pl -locus were the result of recurrent mutation only, one would expect them to occur in different populations in about the same frequency. This was true in most cases, or at least it was not possible to demonstrate any statistically significant differences between human populations with one exception, the Pld_2 -allele. The Pld_2 -allele was found among Negroes and Caucasians, but has so far not been reported among Asiatic mongoloids. It may therefore be maintained by a balanced polymorphism.

The agreement with the expected Hardy-Weinberg distribution was usually good. The large deviations from the Hardy-Weinberg equilibrium found in the Hawaiian population are fully explained by the lack of racial panmixia known to exist in this population (*cf.* Lind, 1955). The more moderate deviations found in the total Swedish material are not, like in the Hawaiian population, characterized by a consistent excess of homozygotes and a deficiency of heterozygotes.

Natural selection

The mechanisms which maintain the placental alkaline phosphatase polymorphism are not known with certainty but the likely existance of immunological incompatibility has already been pointed out. The common phenotypes were found to be associated with the following factors of potential selective significance viz.:

- a) rate of abortion in previous pregnancies;
- b) alkaline phosphatase activity;
- c) placental weight.

The lowest rate of previous abortion was found associated with the most common allele Pls_1 which is in agreement with the

theoretical expectation of a lower selection pressure against frequent genes. The frequency of the F_1 -phenotype was significantly higher ($p = 0.01$) among children whose mothers had had previous abortions. These results are only tentative; definite conclusions will have to await further confirmation. A statistical analysis of the relation between the I_1 -factor and abortion was not attainable due to the rareness of this factor.

The mechanisms of selection against the Plf_1 - and Pli_1 -alleles are probably different for only the Pli_1 -allele was associated with a lower enzyme activity. The effect was rather pronounced, since the activity attributable to the Pli_1 -allele was only about half that of the Plf_1 - and Pls_1 -alleles. The physiological consequences of this activity difference are unknown but it seems likely that at least the low activity found in the I_1 -homozygotes should be disadvantageous.

The placental weight was significantly lower in placentae having the S_1 -factor. One may speculate that a low placental weight may be of some advantage e. g. in situations where the mother is developing antibodies against placental alkaline phosphatase, since there is a clear effect of placental weight on the enzyme level in the mothers' serum.

Physiological role

The physiological roles of the placental acid- and alkaline phosphatases are unknown so far. In this investigation all genetic variants of placental alkaline phosphatase have however been shown to hydrolyze inorganic pyrophosphate as well as nucleotide phosphates. Cox et al. (1967) have proposed that alkaline phosphatase physiologically acts as inorganic pyrophosphatase. Inorganic pyrophosphate is produced in many biosynthetic reactions and Stetten (1960) and Kornberg (1962) have proposed that certain reactions which produce pyrophosphate e. g. nucleic acid synthesis are made irreversible due to the hydrolysis of inorganic pyrophosphate by one or more inorganic pyrophosphatases. The concentration of alkaline phosphatase on the chromosomes during mitosis (Danielli, 1953) is in agreement with the supposed role of alkaline phosphatase in nucleic acid synthesis by hydrolysing pyrophosphate an inhibitory product of the reaction (*cf.* Cox et al. 1967).

S U M M A R Y

1. Six common phenotypes of placental alkaline phosphatase were identified by means of starch gel electrophoresis of placental extracts. The genetics of these phenotypes was studied in a material of 100 twin pairs with dichorionic placentae. The results confirmed that placental alkaline phosphatase was controlled by the foetal genotype and that the six common phenotypes were controlled by three autosomal alleles (Plf_1 , Pli_1 and Pls_1). Homozygous types showed one major electrophoretic zone. Heterozygous types displayed a triple-banded pattern where the zone with intermediate mobility was a hybrid enzyme. The alkaline phosphatase activity attributable to the Pli_1 -allele was estimated to about half that of the Plf_1 - and Pls_1 -alleles.
2. Eleven rare variants of placental alkaline phosphatase distributed on eighteen rare phenotypes were found in an investigation of a total of 4374 placentae from different ethnic groups. The rare phenotypes showed triple-banded heterozygous patterns with the exception of two types (F_3 and S_6) which may be homozygous for rare genes. One particular variant (D_2) displayed electrophoretic properties on starch gel electrophoresis which were independent of electric charge and molecular size and hence most likely due to an absorption to the starch gel medium.
3. In extracts of placental tissue four electrophoretically distinct acid phosphatase isozymes (A, B, C and D) were found. Isozymes A, B and D showed a general somatic distribution while isozyme C was typical of placenta and other foetal organs. Four out of

4374 placentae showed a deviating electrophoretic pattern. Evidence was presented that the C- and D-isozymes of the placenta shared a polypeptide subunit with three acid phosphatase isozymes found in leukocytes.

4. Placental alkaline phosphatase was found regularly in the sera of pregnant women at term. The level was strongly influenced by alkaline phosphatase phenotype and placental weight, but was independent of ABO blood type. None of the placental acid phosphatases were found as serum enzymes.
5. The frequencies of the three common alleles showed marked differences between the main ethnic groups of the world. Pl_{S1} was the most common allele in all populations, but showed its highest frequency among African Negroes. The highest frequency of the Pl_{F1} -allele was found among Caucasians, while the Pl_{I1} -allele was most common among Asiatic mongoloid populations. The eleven rare alleles had a gene frequency below 1 per cent. In most populations a good agreement was found between observed and expected numbers assuming a Hardy-Weinberg equilibrium. The mixed total population of Hawaii formed an exception in this respect.
6. The placental alkaline phosphatase factor S_1 showed a significant association with a lower placental weight and a lower rate of previous abortion ($0.05 > P > 0.01$). The frequency of the F_1 -phenotype was higher ($P = 0.01$) among newborn children whose mothers had had previous abortions.
7. Evidence was presented suggesting that the heat-stable alkaline phosphatase of HeLa cells was identical with the S_1 -variant of placental alkaline phosphatase. This isozyme was not found in fibroblastic cell strains. An acid phosphatase corresponding to the placental isozyme C was found in some fibroblastic cell strains, but not in epithelial cell lines.
8. All genetic variants of placental alkaline phosphatase were able to hydrolyze physiological substrates like inorganic pyrophosphate and nucleotide phosphates. Attempts to demonstrate pyrophosphatase activity of the placental acid phosphatase isozymes were unsuccessful.

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